**Correction to the ICCVAM Test Method Evaluation Report: Current Validation Status of *In Vitro* Test Methods Proposed for Identifying Eye Injury Hazard Potential of Chemicals and Products (NIH Publication No. 10-7553)**

## BCOP False Negative Substances for the EPA and GHS Classification Systems

Two additional false negative substances, L-aspartic acid and DL-glutamic acid, were inadvertently omitted from the ICCVAM evaluation of the BCOP for its accuracy for identifying substances not classified as eye irritants. Accordingly, this document serves to correct the applicable sections and tables in the ICCVAM Test Method Evaluation Report (ICCVAM 2010). The table below replaces Table 2-1 (page 5) and Table 6-10, Appendix C (page C-58). The text below regarding the false negative substances supplements text on page 3.

The BCOP test method incorrectly underpredicted 10 eye hazard substances as substances that do not require eye hazard classification. All 10 substances are false negative using EPA hazard classification criteria. One substance, L-aspartic acid, is false negative using the less stringent GHS hazard classification criteria. L-aspartic acid produced positive GHS scores for corneal opacity and iritis injuries in 2 rabbits, and produced positive EPA scores for corneal opacity, iritis, and conjunctival redness injuries in all 3 rabbits.

Eight of the 10 false negative substances produced corneal opacity in the rabbit studies. One false negative substance (L-aspartic acid) produced opacity with a severity score of 3 *("opalescent areas, no details of iris visible, size of pupil barely visible"),* with injuries that persisted beyond 3 days (see table below).

Two of the 10 false negative substances produced visible damage inside the eye (iritis) that persisted beyond 24 hours. One of these false negative substances (L-aspartic acid) produced iritis injuries that persisted beyond 3 days in 2 animals.

Six of the 10 false negative substances produced conjunctival redness injuries with a severity score of at least 2 ("*diffuse, crimson red, individual vessels not easily discernable*"). Two of these false negative substances (L-aspartic acid and polyalkenylsuccinate ester/amine salt) produced injuries with the highest redness severity score of 3 *("diffuse beefy red")*, with injuries that persisted beyond 3 days.

Two of the 10 false negative substances produced conjunctival chemosis (edema) severity scores of at least 2 *("obvious swelling with partial eversion of the lids")* that persisted beyond 24 hours. One of these false negative substances (EDTA, dipotassium salt) produced a chemosis severity score of 3 *("swelling with lids about half closed"*).

Available data indicate that one of the newly added false negative substances (L-aspartic acid) is at least GHS Category 2, and may be a GHS Category 1 (irreversible damage). While corneal opacity and iritis lesions were still present at 3 days, no data were provided to indicate whether these effects completely reversed by 21 days, which would be necessary to avoid classification as GHS Category 1. The other newly added substance, DL-glutamic acid, is EPA Category III based on one rabbit with a positive score for corneal opacity.

For the EPA classification system, the false negative rate for BCOP for all reference substances classified as EPA Category I, II, and III eye hazards is 6% (10/155). Among the 155 EPA eye hazard substances in the BCOP validation database, 63 are EPA Category I substances, 22 are EPA Category II substances, and 70 are EPA Category III[1] substances.

For the GHS classification system, the false negative rate for BCOP for all reference substances classified as GHS Category 1 or 2 eye hazards is 1% (1/106). Among the 106 GHS eye hazards in the BCOP validation database, 65 are GHS Category 1 substances and 41 are GHS Category 2[2] substances.

**References:**

ICCVAM 2010. ICCVAM Test Method Evaluation Report: Current Validation Status of *In Vitro* Test Methods Proposed for Identifying Eye Injury Hazard Potential of Chemicals and Products. NIH Publication No. 10-7553. Available at: http://iccvam.niehs.nih.gov/methods/ocutox/MildMod-TMER.htm.

[1] Includes 13 substances now identified as “at least EPA Category III”.
[2] Includes 9 substances now identified as “at least GHS Category 2”.

**Table UPDATED[1] BCOP False Negative Substances[2] (in order of corneal opacity severity from most severe to least severe)**

| Substance (Discordant Class Y/N)[3] | *In Vivo* Classification | | | | *In Vivo* Scores | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Corneal Opacity | | Iritis | | Conjunctival Redness | | Conjunctival Chemosis | |
| | EPA | FHSA 20% | FHSA 67% | GHS | N[4] CO | Number of Animals- CO Score <Day Cleared[5] | N[4] IR | Number of Animals- IR Score <Day Cleared[5] | N[4] CR | Number of Animals- CR Score <Day Cleared[5] (*Score to 0*) | N[4] CC | Number of Animals- CC Score <Day Cleared[5] *(Score to 0)* |
| L-Aspartic acid (Y, solid) | **≥III** | Irr | Irr | **≥2** | 3/3 | 1-CO3 <2 days; CO2 >3 days 1-CO1 >3 days 1-CO1 <3 days | 3/3 | 2-IR1 >3 days 1-IR1 <2 days | 3/3 | 3-CR3 <2 days (*2 >3 days; 1 >2 days*) | 0/3 | - |
| Iminodibenzyl (Y, solid) | III | Irr | Irr | NC | 3/3 | 3-CO1 <2 days | 0/3 | - | 0/3 | - | 0/3 | - |
| Dimethylbiguanide (Y, solid) | III | Irr | Irr | NC | 2/3 | 1-CO1 <3 days 1-CO1 <2 days | 1/3 | 1-IR1 <2 days | 2/3 | 2-CR2 <3 days (*1 <5 days; 1< 4 days*) | 0/3 | *[2-CC2 <1 day] (2 < 3 days)* |
| Magnesium Carbonate (Y, solid) | III | Irr | Irr | NC | 2/3 | 1-CO1 <3 days 1-CO1 <2 days | 0/3 | *[2-IR1 <1 day]* | 0/3 | *[1-CR2 <1 day] (1 <2 days)* | 0/3 | *[1-CC2 <1 day] (1 < 2 days)* |
| EDTA, dipotassium salt (Y, solid) | III | Irr | Irr | NC | 1/3 | 1-CO1 <3 days *[2-CO1 <1 day]* | 0/3 | *[2-IR1 <1 day]* | 3/3 | 3-CR2 <2 days (*3 <7 days*) | 2/3 | 1-CC3 <2 days 1-CC2 <2 days *[1-CC2 <1 day] (2 <3 days; 1 <2 days*) |
| Polyalkenylsuccinate ester/amine salt (N) | III | Irr | Irr | U[6] | 2/6 | 2-CO1 <2 days *[3-CO1 <1 day]* | 0/6 | - | 2/6 | 1-CR3 <2 days; CR2 <6 days *[1-CR3 <1 day; CR2 <6 days]* 1-CR3 <2 days *[3-CR3 <1 day; CR2 <2 days]* | 1/6 | 1-CC2 <2 days *[1-CC2 <1 day] (1 < 3 days; 1 < 2 days)* |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | (4 >6 days; 2 <6 days) | | |
| DL-Glutamic acid (Y, solid) | **III** | Irr | FTR | NC | 1/3 | 1-CO1 <2 days *[2-CO1 <1 day]* | 0/3 | *[1-IR1 <1 day]* | 0/3 | - | 0/3 | *[3-CC2 <1 day] (2 <3 days; 1 <2 days)* |
| Compound I (Disinfectant/ Cleaner; Unknown) | III | NL | NL | NC | 1/6 | 1-CO1 <2 days | 0/6 | - | 0/6 | - | 0/6 | - |
| Tween 20 (N) | III | Irr | FTR | NC | 0/4 | - | 0/4 | - | 2/4 | 2-CR2 <2 days *(2 <7 days)* | 0/4 | - |
| Methylcyclopentane (Y, ketone) | III | NL | NL | NC | 0/6 | - | 0/6 | - | 1/6 | 1-CR2 <3 days *(1 <7 days)* | 0/6 | - |

Abbreviations: BCOP = bovine corneal opacity and permeability; CO = corneal opacity; CC = conjunctival chemosis; CR = conjunctival redness; EPA = U.S. Environmental Protection Agency; FHSA = Federal Hazardous Substances Act; FTR = further testing required; GHS = Globally Harmonized System; IR = iritis; Irr = irritant; N = number of animals; NC = Not Classified (as irritant); NL = Not Labeled (as irritant); U = unclassifiable.

[1]Updated *in vivo* classifications in bold font.

[2]False negative substances (shaded here) are those that test as substances not labeled as irritants *in vitro* but are mild, moderate, or severe irritant/ocular corrosives *in vivo*.

[3]Among the false negative substances for which chemical class and/or physical properties could be assigned, 7/9 were from discordant classes that have previously been identified for the BCOP test method (i.e., either solids [false negative] or ketones [false positive]). Chemical class information was unavailable for Compound I.

[4]Number of animals with positive responses, based on meeting or exceeding the minimum severity criteria at 24, 48, or 72 hours. The following scores are considered positive: CO or IR ≥1 or CC or CR ≥2. Therefore, CO or IR scores of 0 and CC or CR scores of ≤1 are considered cleared. One-hour observations are noted as italic font in brackets, but were not used for classification.

[5]Day cleared: 1 day = cleared between 1 hour and 24 hours; 2 days = cleared between 24 hours and 48 hours; 3 days = cleared between 48 hours and 72 hours; 6 days = cleared between 72 hours and 144 hours.

[6]This substance cannot be assigned a GHS classification based on the *in vivo* data because there were not a sufficient proportion of positive animals to fulfill the GHS criteria for ocular hazard classification.